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(56) Documents Cited
EP 0850947 A **EP 0693558 A**
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(54) Abstract Title
Nutritional compositions comprising trehalose for persons suffering from diabetes

(57) A method of nutrition of a person suffering from a disorder of insulin metabolism, such as diabetes, comprises the step of oral administration of a composition comprising trehalose. Preferably, the composition comprises at least 10% by weight of the composition and the composition may be chocolate, hard sweets, biscuits, fondants, jellies, jams, sauces, puddings, syrups, soft drinks, sweet or savoury snack foods, baked foods such as cakes or ice cream. Preferably at least 0.1g of trehalose per kg body weight of the person is administered. The composition may be used for the treatment of a medical condition mediated by insulin metabolism, such as diabetes and in particular type 2 diabetes.

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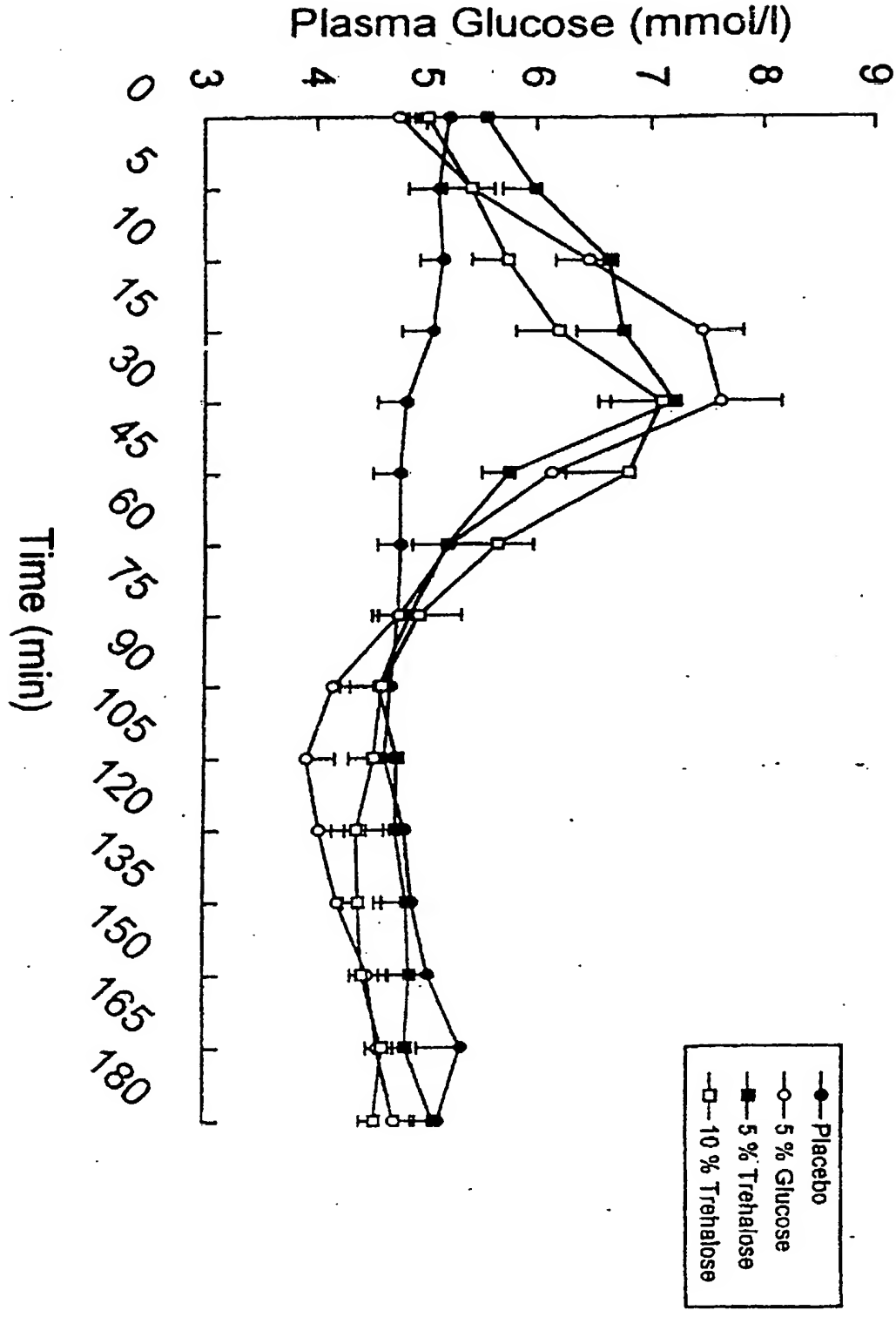
Fig. 1


Plasma Glucose (mmol/l)
3
4
5
6
7
8
9
Time (min)
0
5
10
15
30
45
60
75
90
105
120
135
150
165
180
Placebo
5 % Glucose
5 % Trehalose
10 % Trehalose

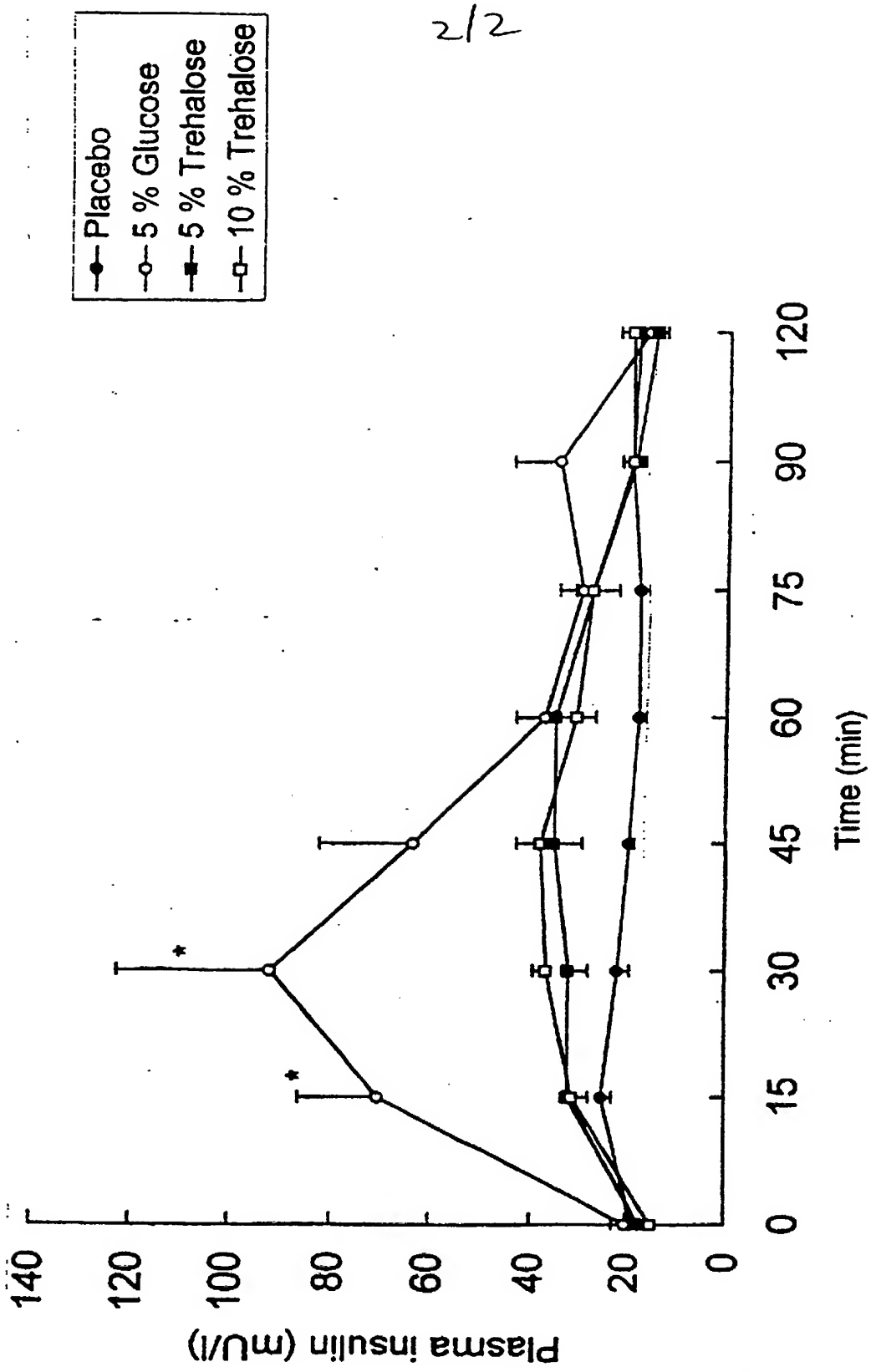

Placebo
5 % Glucose
5 % Trehalose
10 % Trehalose
Plasma insulin (mU/l)
140
120
100
80
60
40
20
0
0
15
30
45
60
75
90
120
Time (min)
*
*

Fig. 2

NUTRITIONAL COMPOSITIONS

The present invention relates to the use of compositions comprising trehalose for the nutrition of persons suffering from medical disorders mediated by insulin metabolism, such as diabetic persons. It also relates to the use of trehalose for the preparations of compositions for use in the treatment or prevention of such disorders.

The short term regulation of blood glucose levels after meals or during the first few hours of starvation is mainly determined by the release of the hormones insulin or glucagon into the blood stream.

The initial high blood glucose levels after consuming carbohydrate-rich foods can often be followed by low blood glucose levels as the released insulin overcompensates, an effect known as a hypoglycaemic dip.

Impairments in the secretion or action of insulin gives rise to increases in blood glucose and can lead to the development of diabetes mellitus. There are two types of diabetes mellitus. Type 1 diabetes is caused by a lack of insulin as a result of damage to the B-cells of the pancreas and is treated with insulin administered by injection. Type 2 diabetes commonly occurs with age and includes defects in the ability of the B-cells to respond to increased glucose concentrations or decreased sensitivity of target cells to normal insulin levels. It has been hypothesised that controlling the incidence of insulin release might reduce the risk or postpone the onset of Type 2 diabetes.

The standard dietetic approach to treating diabetes is to advise patients to reduce the consumption of high glycaemic index foods (i.e. those causing high blood glucose levels), to increase consumption of complex carbohydrates, and to spread their carbohydrate intake throughout the day. However, a balance between carbohydrate intake, other dietary components (mono-unsaturated fatty acids), exercise and insulin dose (Type 1) or response must be maintained to

avoid hypoglycaemia, where blood glucose levels are low and which in severe cases can cause coma.

Trehalose (α-D-glucopyranosyl-α-D-glucopyranoside) is a naturally occurring non-reducing disaccharide found in fungi, certain yeasts, certain drought resistant plants and in the blood of insects. However, it has hitherto contributed an insignificant part of most human diets.

G. G. Birch in Process Biochemistry, July 1970, page 9, briefly reviews the role of trehalose in nature. The author notes that trehalose is quite sweet-tasting, and if it were to be absorbed slowly or not at all by the body, without any ill-effect, it could provide a useful non-fattening dietetic or diabetic sugar.

It has subsequently been established that trehalose is, in fact, rapidly absorbed by the body. The mechanism of absorption is that trehalase enzyme in the microvilli of the small intestine breaks the trehalose down into its constituent glucose monomers, which are then absorbed through the intestinal wall.

WO96/08979 describes isotonic or hypotonic sports beverages containing trehalose. The sports beverages containing trehalose are said to be useful for providing a quick energy boost by restoring blood glucose levels of athletes. The use of trehalose rather than glucose is said to be desirable because of the lower osmotic pressure of a given weight concentration of a disaccharide such as trehalose compared to a monosaccharide such as glucose. There is no suggestion that the sports beverages would be suitable for nutrition of diabetics.

EP-A-0619951 also describes energy supplements containing trehalose. Data are presented showing that relatively small orally administered doses of trehalose (apparently less than 0.01 g/kg of the body weight) boost blood glucose levels slightly more slowly than corresponding doses of pure glucose. It was also found that the trehalose gives a slightly smaller, but still substantial, insulin response than the equivalent amount of glucose. There is no suggestion in this reference to use trehalose in diabetic foodstuffs, and indeed the teaching of the

reference suggests that trehalose should be used to achieve a quick boost in blood glucose levels. The reduction in insulin response of trehalose relative to glucose reported in this reference is insubstantial, and would not lead the skilled person to consider trehalose as a diabetic foodstuff.

The present inventors have found that larger doses of orally administered trehalose behave very differently from the small doses described in EP-A-0619951. Larger doses, such as those conventionally used for nutrition, are absorbed more slowly, and provoke a very much smaller insulin response in healthy subjects than corresponding oral doses of glucose. The reasons for this behaviour are not clear, but it may be due to a limiting rate for trehalose breakdown and transport in the small intestine. In any case, it leads to the surprising conclusion that trehalose may be especially suitable for the nutrition of diabetics and other persons suffering from disorders of insulin metabolism.

Accordingly, the present invention provides a method of nutrition of a person suffering from a disorder of insulin metabolism, comprising the step of oral administration of a composition comprising trehalose.

The above finding also leads to the conclusion that the nutritional use of trehalose may be especially suitable for treatment or prevention of medical conditions mediated by insulin metabolism.

Accordingly, the present invention also provides the use of trehalose for the preparation of a composition for use in the treatment or prevention (prophylaxis) of a medical condition mediated by insulin metabolism.

The treatment referred to is not primarily curative, but rather is directed to reduction of the adverse effects that can arise from conventional nutrition of a person suffering from such a disorder by substitution of the trehalose composition for e.g a sucrose composition. The preventive use of trehalose according to the present invention is envisaged because it is thought that the use of trehalose in

nutrition instead of conventional sugars such as sucrose may reduce the incidence of disorders of insulin metabolism in persons predisposed to such disorders.

The disorder mediated by insulin metabolism may, for example, be diabetes (type 1 or type 2) or hyperinsulinaemia. Conditions leading to increased risk of defects in insulin metabolism include obesity, heart problems, stroke, or physical trauma or disease of organs involved in insulin metabolism. Preferably, the disorder includes diabetes. The use of trehalose according to the present invention may be especially useful for the prophylaxis of type 2 diabetes.

Preferably, the nutritional composition comprises at least 10% by weight of trehalose, more preferably at least 20% by weight of trehalose, still more preferably at least 30% by weight, and most preferably at least 40 or 50% by weight of trehalose based on the total weight of dry substance in the composition.

Preferably, the composition is selected from the group consisting of chocolate, hard sweets, biscuits, fondants, jellies, jams, sauces, puddings, syrups, soft drinks, sweet or savoury snack foods, cakes and other baked goods, ice cream, and combinations thereof. The composition may further comprise an intense sweetener such as saccharin or aspartame to bring the sweetness of the composition up to the level of an equivalent composition made with sucrose.

Trehalose has been found to produce more acceptable diabetic or dietectic food products, for example with improved organoleptic properties or the lack of unpleasant gastro-intestinal effects associated with some carbohydrates such as fructose or sorbitol commonly used in such foods.

Preferably, the step of oral administration comprises administration of at least 0.1g of trehalose per kg body weight of the diabetic person, preferably at least 0.3 g/kg and more preferably at least 0.5g/kg. The benefits of trehalose use in accordance with the present invention are especially notable at the higher doses.

The present invention also provides the use of trehalose for the preparation of an edible composition for use in a method of nutrition of a person suffering from a disorder of insulin metabolism in accordance with the invention.

Specific embodiments of the present invention will now be described further with reference to the following drawings, in which:

Figure 1 shows the effect of ingesting 5% and 10% aqueous trehalose solutions on plasma glucose concentration of healthy (non-diabetic) subjects. Data are also shown for a placebo and for a comparative 5% glucose aqueous solution; and

Figure 2 shows the effect of ingesting the same beverages as in Figure 1 on plasma insulin concentration of healthy (non-diabetic) subjects.

Example 1

Eight healthy male human volunteers, aged 21 ± 1 years (mean ± S.E.M.), body mass 74.0 ± 7.4 kg, height 1.79 ± 0.02 m, body mass index (BMI) 24.0 ± 1.0 k/m^2 volunteered as subjects for the experiment and gave written informed consent. None of the subjects had suffered an illness in the preceding 3 weeks. None of the subjects had recently modified their dietary energy intake and none had undergone marked weight changes in the previous 3 months. Subjects were required to abstain from alcohol intake in the 24 hours preceding each experimental trial. All subjects were given a test drink containing 5 g trehalose prior to commencing the main study. None of the subjects reported any gastrointestinal discomfort following the test drink.

Subjects arrived in the laboratory between 9.00 and 11.00 a.m. following an overnight fast. A 4 cm, 21 g Venflon (registered trade mark) cannula was inserted into an antecubital vein. On four separate occasions subjects consumed 0.7 g per kg body mass of glucose, trehalose or placebo solutions (each solution had the same lemon flavour and contained 20 mmol/l trisodium citrate). Subjects were

asked to consume the drinks within a 3 minute period. The glucose solution contained 5% w/v glucose and two trehalose solutions were used containing 5 and 10% w/v trehalose. The placebo contained saccharin as sweetener but no carbohydrate. Hence, the mean fluid intakes were 1036 ± 104 ml for the placebo (PLA), glucose (GLU) and 5% trehalose (T5) drinks and 518 ± 52 ml for the 10% trehalose (T10) drink. The mean sugar intakes were 51.8 ± 5.2 g for the glucose and trehalose treatments. The osmolality of the drinks was measured using a freezing point osmometer (Advanced Instruments) and was 110, 361, 255 and 386 mOsm/kg for the PLA, GLU, T5 and T10 drinks, respectively (note that 80 mOsm/kg of each drink was attributable to the added trisodium citrate). The order of the treatments was randomised and blinded to the subjects. The subjects remained seated throughout the 3 hours. During each test the subjects were asked about the palatability of the drinks and they were also asked to report any gastrointestinal discomfort experienced during and following the tests.

Blood samples were obtained before consumption of the drink and at 5, 10 and 15 minutes after consumption of the drink. Further blood samples were obtained at 15 minute intervals for up to 3 hours after consumption of the drink. About 5 ml of blood was placed into heparinised tubes. An aliquot was used to determine haemoglobin concentration and haematocrit so that plasma volume changes could be estimated according to Dill and Costill J. Appl. Physiol. Vol 37, pages 247-248 (1974). The remainder of the blood was centrifuged at 1500 g at 4°C to obtain plasma. The latter was stored at –70°C prior to analysis for glucose using an enzymatic, spectrophotometric method (Sigma Chemicals kit HK-20), insulin (by radioimmunoassay using an ICN Biomedicals insulin antibody coated tube kit) and total protein (Biuret method, with bovine serum albumin as standard) concentrations. The coefficient of variation for the assays was ± 1.9% for glucose, ± 0.5% for protein and ± 3.0% for insulin.

Differences with time and treatment were assessed using a repeated measures ANOVA with Tukey post hoc tests where appropriate. The accepted level of significance was $P<0.05$. All data in the text and Figures are reported as

mean ± standard error of the mean (S.E.M.) for eight subjects from whom we obtained the full complement of blood samples.

None of the subjects experienced any gastrointestinal discomfort during or following the tests. Subjects reported that the drinks were palatable.

The mean resting plasma glucose concentration before consumption of the drinks was 5.1 mmo1/1. The change in plasma glucose concentration following consumption of the different drinks is shown in Figure 1. On the PLA treatment the plasma glucose concentration remained stable throughout the 3 hour period. Following GLU ingestion, plasma glucose concentration rose significantly after 10 minutes and reached a peak of 7.64 ± 0.57 mmo1/l alter 32 ± 2 minutes and had returned to normal by 45-60 minutes. After 90-120 minutes the plasma glucose concentration fell to a minimum of 3.59 ± 0.21 mmol/l; at this point the plasma glucose concentration was significantly lower than that observed on the T5 trial.

With the T5 drink, the plasma glucose concentration was significantly higher than on the PLA trial only 5 minutes after consuming the drink. Plasma glucose concentration on the T10 trial was not significantly greater than PLA until 15 minutes alter consuming the drink, but the plasma glucose concentration at this time was still significantly lower than on the GLU trial. After 30 minutes plasma glucose levels began to fall on both the GLU and T5 trials and at 45 minutes were not significantly different from PLA. On the TI0 trial the plasma glucose concentration was higher than PLA after 45 minutes. The area under the curve for the first 60 minutes (AUC60) following consumption of the drinks was not significantly different for GLU compared with either trehalose drink ($P<0.05$). There was, however, quite a large difference between individuals (range 23-97%). As a percentage of the GLU-AUC, the mean values for T5 and T10 were 55 ± 17% and 84 ± 17%, respectively.

Changes in plasma volume were minimal and nonsignificant following all of the drinks and there were no significant differences between the treatments. At the end of the 3 hour period, changes in plasma volume were +3.7 ± 3.2%, +2.2 ±

1.9% +4.0 ±2.6% and +3.7 ± 3.2% for the PLA, GLU, T5 and T10 trials, respectively.

Plasma protein concentration fell on average by 7% during the tests (P<0.0l) but there were no significant differences between the treatments.

The mean resting plasma insulin concentration before consumption of the drinks was 18 mU/l. The change in plasma insulin concentration following consumption of the different drinks is shown in Figure 2. On the PLA treatment the plasma insulin concentration remained stable throughout the 3 hour period. Following GLU ingestion, plasma insulin concentration increased and reached a peak of 92 ± 31 mU/l after 30 minutes and had returned to normal by 90-120 minutes.

With both trehalose drinks, the plasma insulin concentration at 15 and 30 minutes was significantly lower than on the GLU trial (P<0.05) and the peak plasma insulin values observed during the each of the trehalose trials were significantly less than that on the GLU trial (P<0.05; Figure 2). The time to reach peak insulin concentration was longer for the trehalose drinks compared with GLU. The area under the curve for the first 60 minutes (AUC60) following consumption of the drinks was higher for GLU compared with either trehalose drink. As a percentage of the GLU-AUC, the mean values for T5 and T10 were 28% and 30%, respectively.

The results of the study show that trehalose has a glycaemic index that is on average a little lower that that of glucose, but is still medium-high. There was, however, a wide variation between different individuals. Those subjects who had a large AUC60 on T5 also showed a large response on T10, which suggests that interindividual differences in gut trehalase activity may be responsible for this large degree of variation. Plasma glucose concentration was maintained at or above basal (placebo) levels for longer with T10 compared with GLU, and there was no significant reactive hypoglycaemia with T5 or T10, whereas this was noticeable 90-120 minutes after GLU ingestion. This is likely to be due to the substantially

larger peak insulin response following GLU ingestion compared with the trehalose drinks.

Example 2

Foodstuffs in accordance with the present invention are prepared as follows:

(a) Strawberry Flavour Hard Candy.

The candy was formulated as follows:

	%
Trehalose	78.7
Isomalt	19.7
Anhydrous Citric acid	1.0
Strawberry Flavour D4 888	0.3
Strawberry Colour Hexacol 73234	0.3

The hard candy was prepared in accordance with the following instructions:

1. Gently heat trehalose, isomalt and sufficient water to dissolve the sugars.
2. Heat whilst stirring until dissolved.
3. Heat to 180°C.
4. Remove from heat and allow to cool to 130-140°C.
5. Add acid, flavour and colour with stirring.
6. Pour into moulds or stamp using conventional equipment.

(b) Milk Chocolate

A milk chocolate was formulated as follows:-

	%
Trehalose	47
Full cream milk powder (26.5% fat)	20.5

Cocoa butter	17.5
Cocoa mass	12.5
Butter oil	2.0
Lecithin (Topcithin 200)	0.5
Vanillin crystals	0.028

The chocolate was made with conventional chocolate processing machinery in accordance with the following instructions:

1. The trehalose was milled to 50µm.
2. Kneading – Add all the ingredients except the lecithin and enough cocoa butter to provide a suitable consistency for the roller refining operation. Knead for 10 minutes at 40°C.
3. Refining – Standard 5 roll refiners cooled to remove any frictional heat. A single refining operation to give a suitable particle size (25-28µm).
4. Conching – as for making standard milk chocolate. The equipment is set to give a product temperature of 54°C. The remainder of the cocoa butter is added. The material is conched for a minimum of 4 hours.
5. Half an hour before the end of the conching period the emulsifier is added.
6. The chocolate was tempered with the same conditions as for standard milk chocolate (40°C-27°C-30°C) then moulded up.

(c) Tomato Ketchup

A tomato ketchup was formulated as follows:-

	%
Concentrated tomato puree	27.0
Water	24.42
Trehalose	25.0
Distilled malt vinegar	20.0
Starch	2.0
Potassium Chloride	1.28
Salt	0.6

CLAIMS

1. A method of nutrition of a person suffering from a disorder of insulin metabolism comprising the step of oral administration of a composition comprising trehalose.

2. A method according to claim 1, wherein the disorder of insulin metabolism comprises diabetes.

3. A method according to claim 1 or 2, wherein the composition is selected from the group consisting of chocolate, hard sweets, biscuits, fondants, jellies, jams, sauces, puddings, syrups, soft drinks, sweet or savoury snack foods, cakes and other baked goods, ice cream, and combinations thereof.

4. A method according to any preceding claim, wherein the step of oral administration comprises administration of at least 0.1 g of trehalose per kg body weight of the person, preferably at least 0.3 g/kg and more preferably at least 0.5 g/kg.

5. A method according to any preceding claim, wherein the composition comprises at least 10% w/w of trehalose, preferably at least 20% w/w of trehalose, more preferably at least 30% w/w of trehalose, and most preferably at least 40% or 50% by weight of trehalose, based on the dry weight of the composition.

6. Use of trehalose for the preparation of an edible composition for use in a method of nutrition of a person suffering from a disorder of insulin metabolism according to any one of claims 1 to 5.

7. Use of trehalose for the preparation of a composition for use in the treatment or prevention of a medical condition mediated by insulin metabolism.

8. Use of trehalose according to claim 7, wherein the medical condition includes diabetes.

9. Use of trehalose according to claim 7 for the preparation of a composition for use in the prevention of type 2 diabetes.

10. Use according to claim 7 or 8, wherein the composition is selected from the group consisting of chocolate, hard sweets, biscuits, fondants, jellies, jams, sauces, puddings, syrups, soft drinks, sweet or savoury snack foods, cakes and other baked goods, ice cream, and combinations thereof.

11. A method according to any one of claims 7 to 10, wherein the composition comprises at least 10% w/w of trehalose, preferably at least 20% w/w of trehalose, more preferably at least 30% w/w of trehalose, and most preferably at least 40% or 50% by weight of trehalose, based on the dry weight of the composition.

The Patent Office

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INVESTOR IN PEOPLE

Application No:	GB 9921336.5	**Examiner:**	Dr Paul D Jenkins
Claims searched:	1-11	**Date of search:**	20 January 2000

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Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.R): A2B (BMDE9, BSS3)

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Other: Online: EPODOC, JAPIO, TXTEP1, TXTGB1, TXTUS1, TXTUS2, TXTWO1, WPI

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
X	EP 0850947 A1	(HAYASHIBARA) see especially examples B-2 and page 11, lines 34-37	1-11
X	EP 0693558 A1	(HAYASHIBARA) see especially examples B-1 and B-18 and page 29, lines 50-54 and page 33, lines 15-17	1-11
X	EPODOC Abstract CN 1154214 (LAN JIN) see abstract		1-11

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.